REMARKS

The specification has been amended to generic terminology for the trademark products as suggested by the Examiner.

Claims 1, 2, 4, 9, 25, 26, 31, 39 and 43 have been amended to specify that the liquid wash medium is a "liquid wash culture medium." Support for this amendment can be found, for example, in paragraph [0069] found on pages 24-25 of the specification, as well as in Examples 1 and 6.

It is submitted that these amendments do not constitute new matter and their entry is requested.

The Examiner rejected claims 1-9, 11-43 and 45-81 under 35 U.S.C. § 103(a) as being unpatentable over Levee et al. (*Molecular Breeding* 5:429-440, 1999) on the basis of the disclosure in Levee et al. of the regeneration of transformed soft pines, i.e., members of the subgenus *Strobus*. It is submitted that the presently claimed invention is not obvious from the teachings of Levee et al.

In accordance with the claimed invention, enhanced transformation and regeneration of transformed embryonic hard pine tissue is accomplished by minimizing damage to cells subsequent to *Agrobacterium* infection. It was found by the inventors that one technique to minimize damage to cells was to wash cells following *Agrobacterium* infection with a liquid wash medium, a limitation found in the claims. This feature is disclosed in the present specification, e.g. paragraph [0031] on page 9 and in several of the original claims. Damage to cells is minimized by using a liquid wash medium to wash cells subsequent to *Agrobacterium* infection. As discussed in the previous Amendment, distilled water could not be used to wash the cells, but any liquid wash medium could be used. Levee et al. uses distilled water to wash the cells of soft pines following *Agrobacterium* infection.

In the Office Action mailed 8 April 2004, the Examiner contends that "the recitation 'a wash medium' encompasses water given the lack of this term in the description." However, the specification does contain a description of "wash medium." Paragraph [0069] discusses the use of support membranes for transferring cells between "the co-cultivation, wash, and post-wash culture

medium." Thus, the specification describes the wash medium as a "wash culture medium." Furthermore, Example 1 discloses the use of "DCR_4 liquid wash medium" to wash the cells (see paragraph [0063] on page 24), and Example 6 discloses the use of a liquid maintenance medium as the wash medium for washing the cells (see paragraph [0122] on page 49). Thus, the description of the invention clearly discloses that the liquid wash medium does not encompass water. Furthermore, it is submitted that the use of the term "medium" in tissue culture does not mean "water" to a skilled artisan. A skilled artisan readily recognizes that a "medium" contains ingredients, such as nutrients, and is not just water. Thus, the term "medium" is a term of art and means something more than water. Although not to be construed as further limiting the present claims, the claims have been amended to specify that the liquid wash medium is a liquid wash culture medium to provide explicitly what was implicitly covered by this term.

There is no suggestion in Levee et al. to use a liquid wash culture medium and no suggestion that use of a liquid wash culture medium would result in an enhanced transformation and regeneration of transformed embryonic tissue of hard pines. Thus, it is submitted that the claimed invention is not obvious from the teachings of Levee et al.

Furthermore, Levee et al. discloses *Agrobacterium* transformation of white pine, *Pinus strobes*. As is well known in the art, white pine is a soft pine and not a hard pine. As is evident in the name, *Pinus strobes*, white pine is a member of the subgenus *Strobes* and is not a member of the subgenus *Pinus*. For example, most classifications of *Pinus* recognize two major lineages: subgenus *Strobus* (haploxylon or soft pines, with one fibrovascular bundle in the needle) and subgenus *Pinus* (diploxylon or hard pines, with two fibrovascular bundles in the needle). This division is consistent with data from wood anatomy and secondary chemistry, and is supported in recent molecular phylogenetic studies (Strauss and Doerksen, 1990, *Evolution* **44**:1081-1096; Wang and Szmidt, 1993, *Plant Systematics and Evolution* **188**:197-211; reviewed in Price et al., 1998, in *Ecology and Biogeography of Pinus*, Cambridge University Press, Cambridge, pp. 49-68).

Pines have a relatively rich fossil record dating back to the Early Cretaceous, 130 million years ago (review in Axelrod et al., 1986, *Ann Mo Bot Gard* **73**:565-641; Klaus et al., 1989, *Plant Systematics and Evolution* **162**:133-163; Van der Burgh, 1973, *Review of Paleobotany and Palynology*, **15**:73-275; Millar, 1993, *Ann Mo Bot Gard* **80**:471-498). The genetic distance between subgenera, at least between *Pinus* and *Strobus*, may be as large as, or larger than the genetic distance between other conifer genera, e.g., between *Cedrus* and *Abies* (Price et al., 1987, *Systematic Botany*, **12**:91-97), and if strict genetic criteria were used, they should perhaps be treated at generic rank. As is commonly known, hard pines are unable to breed with soft pines, though they can interbreed readily, if the correct timing and other conditions are provided, with other hard pine species (a seminal reference is Critchfield and Little, 1966, *Geographic distribution of the pines of the world*, USDA Forest Service Miscellaneous Publication 991, Washington, D.C.; see also Little and Critchfield, 1969, *Subdivision of the genus Pinus pines*, USDA Forest Service Miscellaneous Publication 1144, Washington, D.C.). Hard pines are unaffected by a number of diseases, such as white pine blister rust, that readily infect soft pines. Their susceptibility to *Agrobacterium* infection appears to be quite different as well.

Levee et al. discloses the transformation and regeneration of pine of the subgenus *Strobus* which, according to this reference, "is the first work on genetic transformation on **this pine species** as well as the first report of successful stable genetic transformation of **a pine species** using a disarmed strain of *A. tumefaciens*". (See page 36, first paragraph of Discussion, emphasis added). Levee et al. does not disclose the transformation and regeneration of pine of the subgenus *Pinus*. The amended claims are clearly directed to pine cells of the *Pinus* subgenus. It is well known to those skilled in the art that somatic embryogenesis systems for soft pines are different from those for hard pines. It is not insignificant that Levee et al. utilized a soft pine which is more easily regenerated than hard pines. Although the Examiner cited art showing transformation and regeneration of soft pine, he has not cited any art showing transformation and regeneration of hard pines as set forth in the claims. Furthermore, it is submitted that there has been no reports in the

literature of the regeneration of plants following stable transformation of embryogenic cultures of any pines of the *Pinus* subgenus by *Agrobacterium*.

Applicants have previously discussed differences between hard and soft pines and the prior inability to regenerate transformed pine tissue of pines of the subgenus *Pinus*, i.e., hard pines, in commercially valuable quantities. One feature of the invention which enables the enhanced transformation and the regeneration of transformed embryonic hard pine tissue is the use of a liquid wash medium as opposed to the use of water to wash cells following *Agrobacterium* infection or cocultivation of embryonic hard pine tissue with *Agrobacterium*. This feature of the invention is found in the claims. The differences between hard and soft pines leads to the unobvious nature of plant transformation and regeneration in these species. In accordance with the Examiner's earlier suggestion that Declarations be submitted to address this point, Applicants are submitting concurrently herewith Rule 132 Declarations of Dr. Marie B. Connett-Porceddu, Mr. David S. Canavera and Dr. James E. Mann.

The Declaration Under Rule 132 of Marie B. Connett-Porceddu (hereinafter the "Connett Declaration") describes the known differences between hard pines and soft pines and the unobviousness of the method claimed in the present application. Specifically, Dr. Connett-Porceddu states that the present invention is directed to the enhanced transformation and regeneration of transformed embryogenic pine tissue in which the pine is of the genus *Pinus*, subgenus *Pinus*, which are the hard pines. *See* Paragraph 6 of the Connett Declaration. Dr. Connett-Porceddu also states that it was discovered that hard pines could be transformed and regenerated to produce transgenic hard pine plants using the disclosed and claimed method of the present application. *See* Paragraph 6 of the Connett Declaration. The present invention allowed for the first time *Agrobacterium*-transformation followed by regeneration of transgenic hard pine plants at a significant frequency. *See* Paragraph 6 of the Connett Declaration.

Dr. Connett-Porceddu states that the cited prior art (Levee et al.) discloses the transformation and regeneration of pine of the subgenus *Strobus* which the authors characterized as the first report

of the successful stable genetic transformation of a pine species. However, this prior art does not show the transformation and regeneration of pines of the subgenus *Pinus*, and a skilled artisan would not expect that the method for soft pines (subgenus *Strobus*) could be used or routinely modified for use with hard pines (subgenus *Pinus*). *See* Paragraph 7 of the Connett Declaration.

To support her latter statement, Dr. Connett-Porceddu states that it was known at the time of the present invention that there were differences between soft pines and hard pines as seen in transformation and regeneration methods for soft pines and hard pines, such that there were no expectation of success with respect to the transformation of hard or soft pines on the basis of the other. *See* Paragraph 8 of the Connett Declaration. Dr. Connett-Porceddu describes the differences between hard and soft pines in Paragraph 9 of her Declaration, including their classification and different susceptibility to diseases and *Agrobacterium* infection. Additional differences between hard and soft pines (a) has been shown for somatic embryogenesis of hard and soft pines as shown by Klimaszewska et al. (attached as Exhibit 4 to the Connett Declaration) and (b) is well known in the art as shown by the Declaration Under Rule 132 of Dr. Micahel Becwar (attached as Exhibit 5 to the Connett Declaration). *See* Paragraph 10 of the Connett Declaration. (See discussion below concerning Exhibit 5.)

Dr. Connett-Porceddu states that (a) there had been no reports of the regeneration of transgenic plants of hard pines (i.e., pines of the subgenus *Pinus*) prior to the present invention and (b) any reports at all concerning regeneration of transgenic hard pines demonstrated that regeneration was not achieved (e.g., Wenck et al., attached as Exhibit 6 to the Connett Declaration). *See* Paragraph 11 of the Connett Declaration. Dr. Connett-Porceddu also states that it is noteworthy that the cited Levee et al. prior art did not discuss at all the regeneration of transgenic plants of hard pine which is the most economic species of conifers. *See* Paragraph 12 of the Connett Declaration. Dr. Connett-Porceddu also states that there have no reports of the application of the method of Levee et al. to the regeneration of transgenic hard pines and in fact, Levee himself has not continued use of the disclosed method for even soft pines. *See* Paragraph 12 of the Connett Declaration. Dr.

Connett-Porceddu further states the assignee of the present application has tried to use or modify the method described by Levee et al. for the regeneration of transgenic hard pine but has not been successful. *See* Paragraph 12 of the Connett Declaration.

Dr. Connett-Porceddu states that experiments had been underway at the assignee of the present application for more than 10 years to adapt systems for regeneration hard pines and for transforming and regenerating transformed hard pines. She states that somatic embryogenesis systems had been developed which worked well with hard pines, but not with transgenic hard pines. *See* Paragraph 13 of the Connett Declaration. The inability to adapt systems developed for transgenic soft pines to transgenic hard pines is further evidence of the differences between soft pines and hard pines and is evidence of no expectation of success in the art for using systems for transgenic soft pines for regenerating transgenic hard pines. *See* Paragraph 13 of the Connett Declaration. Since (a) a person of ordinary skill in the art knew that there were differences between soft pines (subgenus *Strobus*) and hard pines (subgenus *Pinus*) with respect to tissue culture, regeneration and transformation and (b) there was a lack of application of methods between the soft and hard pines, there was no expectation of success in the art for regenerating transgenic hard pines on the basis of a single report for the regeneration of transgenic soft pines. *See* Paragraph 14 of the Connett Declaration.

Exhibit 5 to the Declaration Under Rule 132 of Marie B. Connett-Porceddu is a copy of the Rule 132 Declaration of Dr. Michael R. Becwar submitted in companion application Serial No. 09/973,089 (hereinafter the "Exhibit 5 Becwar Declaration") that explains differences between hard and soft pines and expectations of skilled artisans with respect to working with these conifers based on his experience. *See* Paragraph 10 of the Exhibit 5 Becwar Declaration. Dr. Becwar states that he has worked with both soft pines, particularly *P. strobus*, and hard pines including *P. taeda*, and that his group was the first to report obtaining somatic embryogenic cultures for a soft pine. *See* Paragraph 10 of the Exhibit 5 Becwar Declaration. He also states that although there are similarities in the stage of culture initiation and general appearance of embryogenic cultures of *P. strobus* and

hard pines such as *P. taeda*, the similarities end there. *See* Paragraph 10 of the Exhibit 5 Becwar Declaration. He states that it is generally known and well accepted by those skilled in conifer somatic embryogenesis that what works for one group of pines will not work with another group of pines and provides examples to support his statement. *See* Paragraph 10 of the Exhibit 5 Becwar Declaration. Finally, he states that the knowledge and acceptance in the art that what works with one group of pines will not necessarily work with another group of pines has been his experience in his work with soft and hard pines. *See* Paragraph 10 of the Exhibit 5 Becwar Declaration.

The Declaration Under Rule 132 of David S. Canavera (hereinafter the "Canavera Declaration") describes the long felt need in the art for the transformation and regeneration of transformed tissue of pines of the subgenus *Pinus*, i.e., hard pines. Specifically, Mr. Canavera states that (i) hard pines, particularly loblolly pine (*Pinus taeda)* and including *P. rigida* and *P. radiata* are the most commercially important pines and (ii) transformation followed by reliable regeneration of hard pines is more desirable than the transformation and regeneration of other conifers, such as white pines (subgenus *Strobus*) and spruces. The transformation of hard pines has turned out to be more difficult than for other conifers. *See* Paragraph 6 of the Canavera Declaration.

Mr. Canavera states that researchers have been attempting to transform hard pines with *Agrobacterium* followed by reliable regeneration since about 1988-1989. He also states that although *Agrobacterium* transformation of hard pines had been achieved and regeneration of hard pines had been achieved, regeneration of *Agrobacterium*-transformed hard pines had not been achieved despite considerable effort. *See* Paragraph 7 of the Canavera Declaration. These facts are corroborated by the references included as Exhibits 1 and 2 to the Canavera Declaration and the dearth of published reports on regenerated transformed hard pines. *See* Paragraph 7 of the Canavera Declaration. Thus, Mr. Canavera states that there was a long-felt need to develop (i) improved methods of *Agrobacterium* transformation of hard pines and improved selection of transformed tissue and (ii) methods to regenerate *Agrobacterium*-transformed hard pines. This long felt need was not satisfied by transformation of other conifers. *See* Paragraph 7 of the Canavera Declaration.

Mr. Canavera further states that the method for hard pine transformation and regeneration described and claimed in the present application and the method for selection of transgenic Southern yellow pine tissue described and claimed in companion application Serial No. 09/973,089 are the first methods that achieved reliable and efficient regeneration of transgenic hard pine plants. The reliable and efficient regeneration of transgenic hard pine results directly from the methods described and claimed in these applications. *See* Paragraph 8 of the Canavera Declaration. Mr. Canavera also states that these methods are sufficiently robust to fill the long-felt need because the methods have been shown to be valid for a wide variety of genotypes of hard pines, a result which had not been achieved without these methods. *See* Paragraph 8 of the Canavera Declaration. Thus, Mr. Canavera concludes that the methods claimed in Serial Nos. 09/973,088 and 09/973,089 meet the long-felt need of providing regenerated *Agrobacterium*-transformed hard pines. *See* Paragraph 8 of the Canavera Declaration.

Finally, Mr. Canavera states that a method such as one used by the Canadian Forest Service (Levee et al.) that is not able to be used for multiple species did not meet the long felt need for the regeneration of transgenic hard pines, whereas the method described and claimed in Serial No. 09/973,088 does satisfy the long-held need. *See* Paragraph 9 of the Canavera Declaration. Mr. Canavera also states that a method that did not enable selection even from within elite families of loblolly pine would not meet the long felt need, whereas the method described and claimed in Serial No. 09/973,089 does satisfy the long-held need. *See* Paragraph 9 of the Canavera Declaration.

The Declaration Under Rule 132 of James E. Mann (hereinafter the "Mann Declaration") describes the commercial success of the present invention. Specifically, Dr. Mann states that hard pines, particularly loblolly pine (*P. taeda)* and including *P. rigida* and *P. radiata* are the most commercially important pines. *See* Paragraph 6 of the Mann Declaration. Dr. Mann also agrees with Mr. Canavera that (i) there was a long felt need for the transformation and regeneration of transformed tissues of pines of the subgenus *Pinus* (i.e., the hard pines) and (ii) that this long felt

need was not satisfied by the transformation of other conifers, such as white pines and spruces. *See* Paragraph 7 of the Mann Declaration.

Dr. Mann also states that his employer, ArborGen, desired a robust, commercializable system for repeatable, reliable pine transformation followed by efficient selection and embryo development/formation. Dr. Mann states that ArborGen licensed the present application and companion application Serial No. 09/973,089, because suitable protocols for such a system did not exist elsewhere. *See* Paragraph 8 of the Mann Declaration. Dr. Mann further states that AborGen is actively using the methods claimed in these applications and have transformed plants in field trials. *See* Paragraph 9 of the Mann Declaration. Finally, Dr. Mann states that AborGen has been approached by other researchers desiring to enter into deals with ArborGen so that AborGen would use the methods described in these licensed patent applications to prepare transgenic hard pine plants with genes of interest to these researchers. According to Dr. Mann, this fact is further evidence of the commercial value of the methods claimed in Serial Nos. 09/973,088 and 09/973,089. *See* Paragraph 10. Thus, Dr. Mann concludes that these facts demonstrate the commercial success of the methods claimed in these applications. *See* Paragraph 11 of the Mann Declaration.

It is submitted that the Rule 132 Declarations filed concurrently herewith establish that the present invention is not obvious from the teachings of Levee et al. It is further submitted that the use of a liquid wash culture medium to minimize damage to cells following co-cultivation with *Agrobacterium* is not obvious from the teachings of Levee et al. Thus, it is submitted that the claimed invention is not obvious from the teachings of Levee et al. Withdrawal of this rejection is requested.

The Examiner provisionally rejected claims 52-57 under the judicially created doctrine of obviousness-type double patenting over claims 1, 3 and 33-37 of copending U.S. application Serial No. 09/973,089. Applicants submit herewith a Terminal Disclaimer to obviate this rejection. Withdrawal of this rejection is requested.

In view of the above amendments and remarks, in conjunction with the remarks made in the previous amendments, it is believed that the claims satisfy the requirements of the patent statutes and are patentable over the prior art. Reconsideration of the instant application and early notice of allowance are requested. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

Respectfully submitted,

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